MORTALITY OF THE COD EGG IN RELATION TO TEMPERATURE

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The general fact that the rate of development of fish eggs becomes greater as the temperature is increased is well known, but knowledge of the relative mortality of the eggs at different temperatures is poor. In nature, the extent of mortality of the egg is particularly important in the case of marine fish which produce pelagic eggs because such eggs may be carried by currents from the spawning grounds into regions of different temperature. The location in which the majority of the eggs will ultimately hatch depends not only upon the rate of development but more upon the relative mortality at various temperatures encountered. (*Cf.* Bigelow, 1926; Fish, 1927.) Conversely, in cases where developing eggs or larvae are taken in plankton hauls, the site at which the eggs were spawned may be traced provided the rates of development and mortality are known and the velocity and direction of the current and temperature of the water can be ascertained. (*Cf.* Walford, 1938; Jacobsen and Johansen, 1908.)

The experiments described in this paper were undertaken to test the extent of mortality in the cod egg at various temperatures and to determine the highest temperature at which development is possible for this species.

Dannevig (1894), who made the first comprehensive attempt to find the temperature range for the cod, was able to hatch eggs from 0° to 14° C. Johansen and Krogh (1914), the most recent investigators in this field, have confirmed this pioneer work. Although chiefly interested in the temperature coefficient, they carried out experiments to establish the upper temperature limit, which they placed at 10.2° C., as eggs hatched at this temperature but failed to do so at 12°, 13°, 16.5° and 20° C.

Investigators who have been more directly concerned with mortality in fish development are Drouin de Bouville (1908), Hein (1907, 1911), Hata (1927), and Rollefsen (1932). Hein subjected trout eggs to adverse conditions at various times after fertilization and demonstrated

[1] The author wishes gratefully to acknowledge the advice and encouragement of Dr. George L. Clarke of Harvard University.

that variations in mortality occurred in relation to the length of time development had proceeded. Rollefsen has confirmed these results, particularly for the early stages of development, and has correlated the decrease in susceptibility to mechanical shock, with the closing of the blastopore.

Procedure

The cod egg was chosen for these experiments because it normally floats at the surface of the water while alive and sinks when dead or dying, thus presenting a clear-cut criterion of death. Since the chorion is transparent, an unhampered view of the embryo is afforded and consequently the stage of development may be easily determined. Material was readily obtained through the courtesy of the Gloucester Station of the United States Bureau of Fisheries.

Eggs were obtained from cod taken in Ipswich Bay, Massachusetts, on March 31, 1937. The surface temperature was 2.5° C. The eggs were transported in insulated jugs at 2.5–3° C., to the laboratory where they were apportioned into beakers at the different constant temperatures. Eight hours had elapsed since fertilization but due to the low temperature the eggs were only just past first cleavage. (Plate I, Fig. 2.)

The 500-cc. beakers used were open at the top, and only 200 cc. of aerated sea water were introduced in order to provide a relatively large surface area for gas exchange. The beakers were placed on racks in five constant temperature tanks maintained to within 0.3° C. at 6, 8, 10, 12, and 14° C. respectively, by means of thermo-regulators. The first four tanks were placed in a cold room at 2° C. and each appropriately heated. The fifth tank was kept in a laboratory at room temperature and cooled to the desired temperature by means of a Kelvinator refrigerating unit.

The sea water, which had a salinity of 32.31‰, had been obtained at Nahant, Massachusetts, six months previously and allowed to stand. During this period all organic matter had decomposed and fouling of the water during the experiment was reduced to a minimum. Although this decomposition process reduced the oxygen content so that thorough aeration of the sea water was necessary before use, it was not necessary to aerate the jars containing the eggs during the experiment.

The eggs were distributed among twenty beakers and four beakers were placed in each of the five tanks. Each day the eggs were transferred to fresh beakers containing water that had been prepared in advance and adjusted to the proper temperature. Transfer was accomplished by means of a scoop made of fine mesh nickel screening.

The eggs which sank to the bottom—and hence were judged to be dead—were removed daily from each jar by pipette, counted and preserved. The fixative used was Stockard's solution [2] which turns the embryo white and leaves the yolk clear. This facilitates the determination of the degree of development in preserved material.

In many cases the eggs were examined before preservation and there was no doubt that they were either dead or dying.

The live eggs in two of the beakers (jars *A* and *B*) in each tank were sampled daily in small numbers to determine the stage of development. They were examined before preservation and the stage carefully noted according to the plan presented in Plates I and II. In every sample the eggs were all found to be very near the same stage. In a few cases in which the stage of a small number departed noticeably from that of the majority, the embryos were obviously monsters or aborted forms whose development could not have proceeded much further. These abnormal forms were encountered more frequently during the early days of development than later.

This daily sampling of jars *A* and *B* resulted in a continued reduction of the total numbers of eggs. Since it was feared that this change in concentration might influence the mortality, parallel tests without sampling were made in the third and fourth beakers in each tank (jars *MA* and *MB*). Only dead eggs were removed in the case of these two beakers. However, the mortalities which resulted in the jars that were sampled turned out to be substantially the same as those obtained for jars that were not sampled.

Rate of Development and Drift at Sea

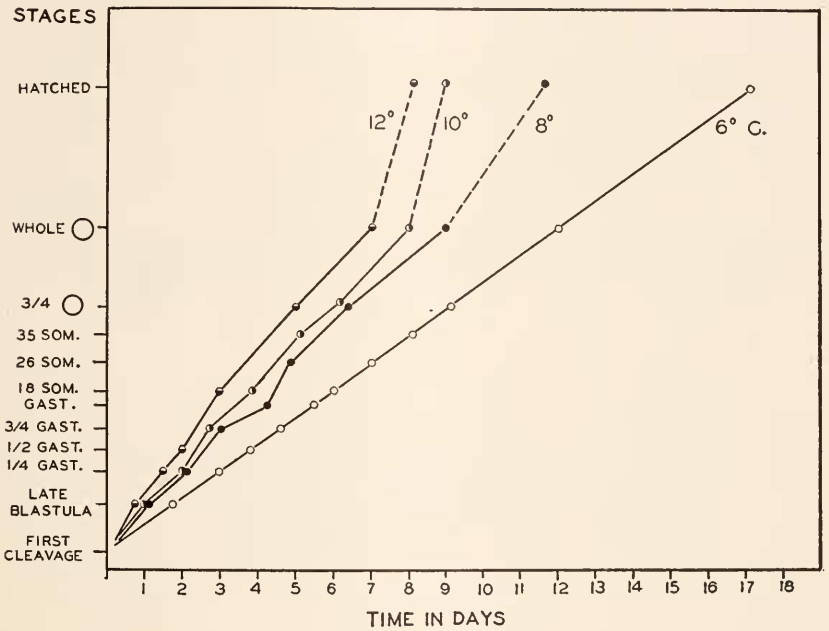
The considerable lapse in time between spawning and hatching for the various temperatures tested may be appreciated from the curves plotted in Fig. 1. The tests run at 14° showed no survival after 24 hours and a subsequent test at 13° also failed. Hence, 12° C. was concluded to be very near the upper limit of temperature for the development of the cod egg. The low survival at this temperature and previous work of Dannevig (1894) and Johansen and Krogh (1914) are in accord with this view.

The young fry of the cod are believed to take to the bottom 7 days after hatching according to McIntosh and Masterman (1897). It is of interest to calculate the point to which Ipswich Bay cod eggs will drift and to ascertain whether the young will find themselves in a suitable locality when they are ready to take to the bottom. According to Fish

[2] Stockard's solution consists of 5 parts 40 per cent formalin, 6 parts glycerin, 4 parts glacial acetic acid, and 85 parts distilled water.

(1927) most of the eggs produced in Ipswich Bay do not drift into Massachusetts Bay but are carried off to the southward. These eggs may be supposed to drift at a rate similar to those leaving Massachusetts Bay, which were shown by Fish to move about 3 or 4 miles per day. At 6° development to hatching required 17 days. With 7 days added to reach the bottom, the eggs would drift 72 to 96 miles.

It is probable that the temperature at which the Ipswich Bay eggs develop is considerably lower than this. Bigelow and Welsh (1925)



TEXT FIG. 1. The development of the cod egg at four different constant temperatures. Abscissa: Time in days. Ordinate: Stage of development determined by the 6° C. which is arbitrarily plotted as a straight line. The curves for the other temperatures plotted against the stages so determined.

state: "The chief production of cod eggs is during the cold months, on the Ipswich grounds, for example, ripe fish are taken when the bottom water is still as warm as 6.6 to 7.7° C. in early September, but they appear in great numbers in temperatures of 5° to 6° C. in January, and as the breeding progresses, the temperature falls, spawning being at its height in the minimum temperature of the year (March), that is at 0.5° to 3.0° C." Poulsen (1931), McKenzie (1934), and others agree that cod may spawn at temperatures ranging from 8° down to 0° C. At a temperature of 3° C. hatching occurs in 23 days according to

Dannevig (1894). With 7 days added for larvae to take to the bottom, at a maximum of 4 miles per day the egg would travel 120 miles from its spawning ground. In the present case such a journey would carry the egg to the northwestern edge of Georges Bank. This accords well with Fish (1927) that the eggs spawned in inshore areas such as Ipswich Bay take to the bottom on Georges Bank.

We may next inquire whether the temperature in this region during the critical months ever becomes sufficiently high to result in serious mortality to the cod eggs. Bigelow (1927) has shown for the Gulf of Maine that the temperatures which prevail during the spring months and early summer are well below 12° C. During February and March the highest surface temperature recorded was 5° C., while during April, the temperature at the surface was below 6° C. In May, the waters have warmed to 10° C. in a few places, but the temperature does not rise above 12° C. until the latter part of July and during August. Inasmuch as this is well after the eggs have hatched, mortality due to too high temperatures is not normally a factor limiting the distribution of the developing cod egg from the Ipswich Bay spawning grounds.

Observations on Mortality

Sources of Error

The principal source of error in this experiment was the use of the sinking of the egg as a criterion of death. On several occasions it was noted in sampling the floating eggs that there were some eggs, particularly among the early stages, which were only masses of cells without

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Stages in the development of the cod. Magnification 30 ×. Figures marked with (*) were used as stages of development.

Fig. 1. Protoplasmic cap, prior to first cleavage. Drawn from material preserved 15 minutes after fertilization.

Fig. 2*. Two-cell stage. Ten hours after fertilization at 0°–3° C. Stage at distribution of eggs.

Fig. 3. Eight-cell stage, polar view. Twenty-eight hours after fertilization at 0°–3° C.

Fig. 4. Early blastula, 52 hours after fertilization at 0°–3° C.

Fig. 5. Middle blastula. Seventy-six hours after fertilization at 0°–3° C. Twenty-four hours after Fig. 2 at 6° C.

Fig. 6*. Late blastula. One hundred hours after fertilization at 0° C. Twenty-four hours after Fig. 2 at 10° C.

Fig. 7. Early gastrula. Forty-eight hours after Fig. 2 at 6° C.

Fig. 7*a*. Ibid. Polar view.

Fig. 8*. One-quarter gastrula. Fifty-two hours after Fig. 2 at 6° C.

Fig. 9*. One-half gastrula. Seventy-three hours after Fig. 2 at 6° C.

Fig. 9*a*. Ibid. Eccentric polar view.

Fig. 10*. Embryo beyond the three-quarters gastrulation stage; 120 hours after Fig. 2 at 6° C. First specks of pigment appearing.

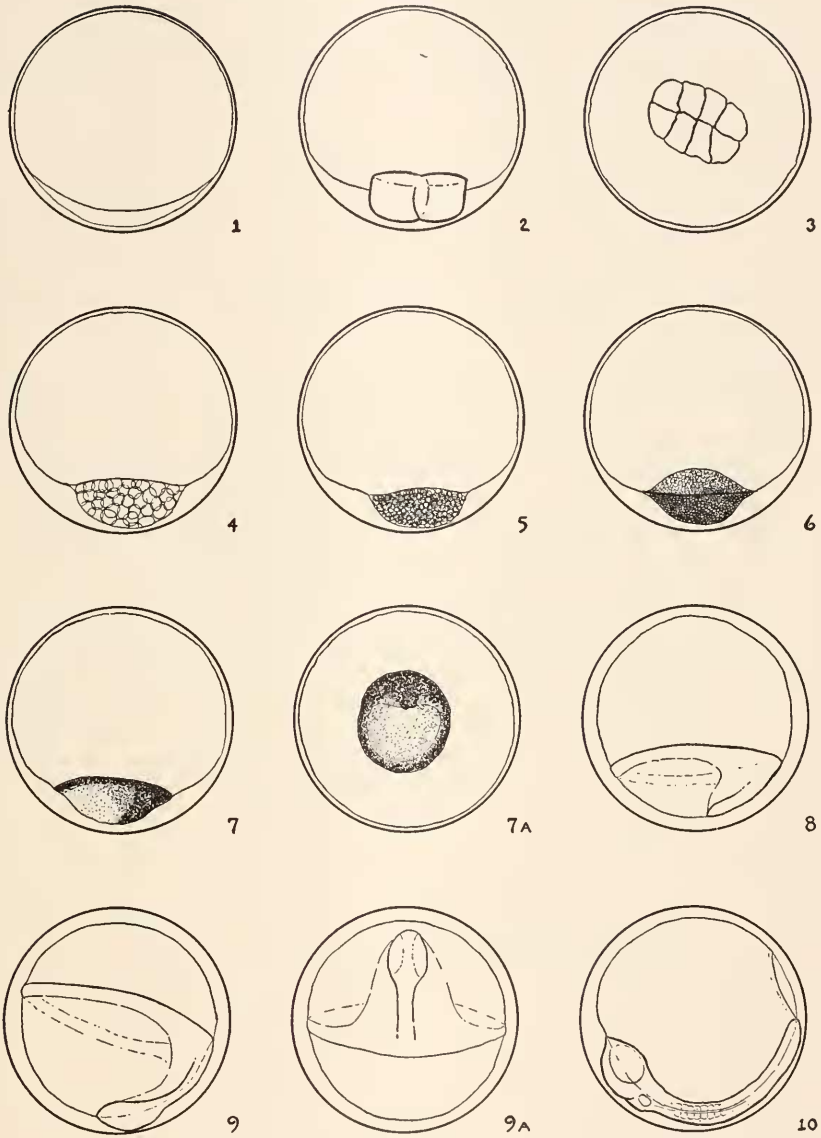

PLATE I

any morphological features to determine the stage. Brice (1898) states that floating eggs are not necessarily alive, for unfertilized and injured eggs usually float for 18 to 36 hours before going to the bottom. In dealing with daily mortality, one must recognize the possibility of a lag of this duration. Observations on such aborted eggs indicated that the number at any time was negligible in relation to the total number which sank during 24 hours.

That some eggs which sink may be alive is likewise a possibility. In fact, McIntosh (1893) states that in certain of his experiments with the cod, the eggs retained their vitality although they did not float. But in the present experiment, examination of the non-floating eggs showed that less than 1 per cent appeared normal. Furthermore, it was observed that death soon followed when the eggs sank, although development did continue for a short time.

During the later stages of development this criterion becomes less certain, for Jacobsen and Johansen (1908) have shown that the specific gravity of the egg becomes greater as development proceeds. It was observed that at 6° C. all the eggs floated at the surface until the tenth day after which the eggs became scattered in various layers in the water. This increased the difficulty of removing only dead eggs. Therefore from the tenth day opaqueness of the egg was used as the criterion of death. Since only about 2 per cent of eggs removed contained embryos with beating hearts, important numbers of live eggs were not being removed with the dead eggs. Although a certain number of dead eggs which had not yet become opaque were probably missed, the effect

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Stages in the development of the cod. Magnification 30 ×. Figures marked with (*) were used as stages of development for graphs.

Fig. 11*. Just prior to whole gastrula or final closing of the blastopore; 126 hours after two-cell stage at 6° C. (Plate I, Fig. 2.) Embryo of 11 somites. Apolar view.

Fig. 12*. Embryo of 18 somites; 144 hours after two-cell stage at 6° C. Pigment definitely appearing.

Fig. 12*a*. Ibid. Apolar view. Pectoral limb buds forming.

Fig. 13*. Embryo of 35 somites. Tail twisting to the right; 192 hours after two-cell stage at 6° C.

Fig. 14*. Three-quarters circle. Apolar view. Heart beginning to beat. Muscular movements of the tail; 216 hours after two-cell stage at 6° C.

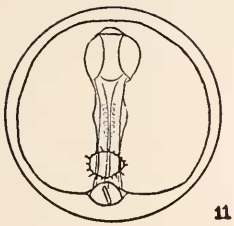
Fig. 14*a*. Ibid. Lateral view.

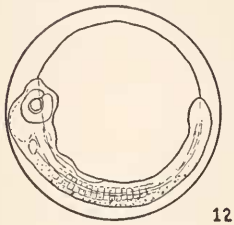
Fig. 15*. Whole circle. Tail approximates head. Upper part of eye well pigmented; 288 hours after two-cell stage at 6° C.

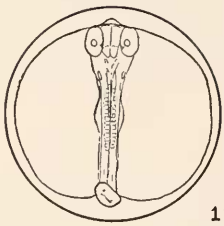
Fig. 16. Just prior to hatching. Pigment localized into definite bands. Eye well pigmented; 396 hours after the two-cell stage at 6° C.

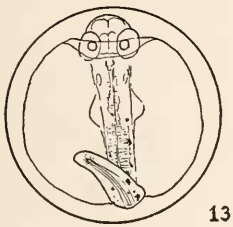
Fig. 17. Lateral view of head of Fig. 16.

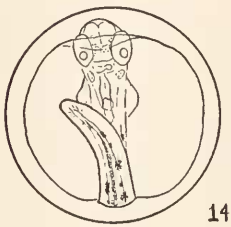
Fig. 18. Larval cod about three days after hatching. Floats with yolk-sac uppermost during first two days after hatching at 6° C.

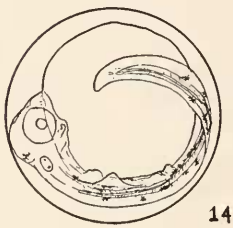


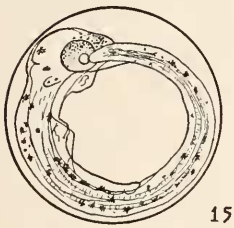


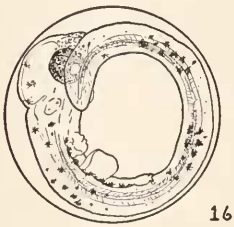


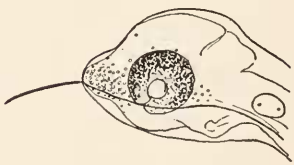












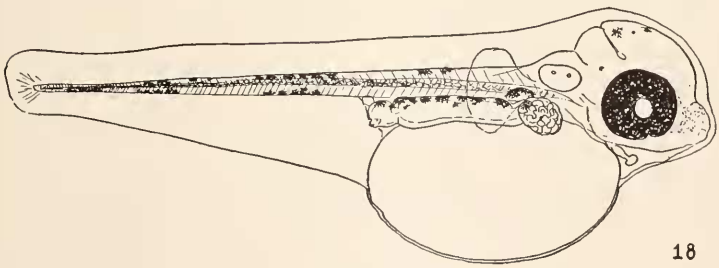


PLATE II

of this procedure merely introduced a delay in the time when those eggs would be counted as dead. The high mortality which occurred in these experiments does not interfere with an interpretation of the results, for the interest lies not in the number of deaths but in the distribution of mortality in relation to the stage of development.

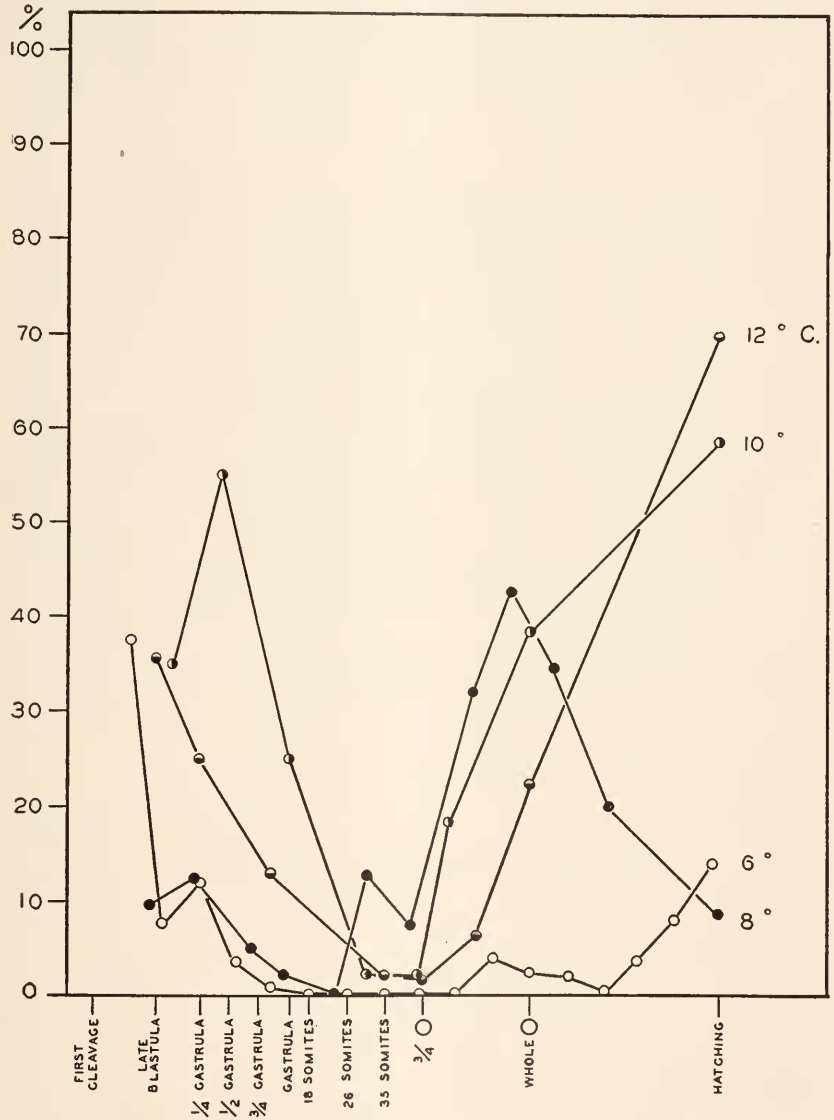
Results of Experiments

The time required for the eggs to reach various stages at different temperatures may be observed in Fig. 1, in which the data obtained from daily sampling have been plotted following the method used by Worley (1933) for the mackerel. Those stages in development of the cod which have been used for this purpose are indicated in the legends of Plates I and II. The stages plotted are not of equal duration, as they were chosen on the basis of certainty of recognition. They have been spaced on the graph in such a way as to make the 6° C. development approximate a straight line. The development of the eggs at the other temperatures was then plotted against these same ordinates.

Confusion has existed in the past over the use of "hatching" as a stage in development because the times when the first and last egg hatch are ordinarily observed directly whereas in the case of the other stages, the samples of eggs withdrawn for microscopic examination allow only the modes of the stage to be determined. Obviously, if the time for each of the earlier stages is taken as the mode for the whole population, then the time designated for the hatching stage should be the point at which the largest number of eggs hatch. Merriman (1935), in his work on the cut-throat trout, however, has taken the time to "first hatching" as one stage and the "eyed-out" condition as another stage. This procedure is fallacious unless the moment when the first embryo entered the eyed-out stage was observed. But even if this were done, the method gives a poorer measure of the rate of development for the whole population than the use of the mode.

Merriman objects to employing the middle of the hatching period as the time to "hatching" because he finds that the "*duration*" of the hatching period (i.e. the time from the hatching of the first egg to the hatching of the last) varies with the temperature in a different manner than the duration of the whole period of development. But the amount of "spread" of the eggs in any stage has no necessary connection with the changes in average time for complete development at different temperatures. Furthermore, in stages other than "hatching" the time that each egg remains in a given stage may be affected differently by temperature at the various points of development. The influence of tem-

perature on the total time for development, and on the time elapsed in each stage should therefore be carefully distinguished. In the present case the information desired is the average total time for development



TEXT FIG. 2. The percentage mortality of the cod egg in relation to stage of development. Abscissa: Stage of development. Ordinate: Percentage daily mortality. (The number of eggs that died during any day divided by the number of eggs that were alive at the beginning of that day multiplied by 100.)

involving a summation of the average times in each of several stages. Therefore, the time taken should be the time required for the "modal" or typical egg at each temperature. Worley (1933) accomplished this by means of frequency distribution. In the present experiment, the number hatching during each day was observed. The time of "hatching" was taken as the day when 50 per cent of all the eggs that hatched during the entire hatching period had completed their development in

TABLE I

Percentage of eggs hatching at different constant temperatures

Temperature	Jar	Total no. of eggs	Number hatched	Percentage hatched of total eggs	Average
° C.				*per cent*	*per cent*
6	IA	1653	10	0.6	11.2*
	IB	2059	8	0.4	
	IMA	118	20	16.9*	
	IMB	546	148	27.1*	
8	IIA	1913	84	4.3	3.3
	IIB	2975	124	4.1	
	IIMA	1811	70	3.8	
	IIMB	1509	15	1.0	
10	IIIA	1820	60	3.2	1.9
	IIIB	339	7	2.0	
	IIIMA	1638	6	0.3	
	IIIMB	1730	37	2.1	
12	IVA	2831	2	0.07	0.04
	IVB	2837	0	0.0	
	IVMA	2312	0	0.0	
	IVMB	1690	2	0.12	

* These values are not comparable to the other figures since the initial number of eggs is very low and the factor of crowding must be considered.

the egg stage,—a method not quite as accurate but more convenient in the case of a slowly developing egg.

A comparison of the curves of Fig. 1 reveals the fact that when the stages are so arranged that a straight line results at 6° C., approximately straight lines are obtained for the other temperatures. This means that the effect of different temperatures on the rate of development of the modal egg was relatively the same in all stages. At 6° C. development was almost twice as long as the development at 12°. This compares favorably with the results obtained by Dannevig (1894) and Johansen and Krogh (1914). The difference between 10° and 12° is much less

than that between 6° and 8° although the temperature change in both cases is 2° C. The explanation lies in the fact that we are dealing with the upper part of the temperature range, the optimal temperature being about 6.5° C., and as the maximum is approached there is a retardation of development. Worley (1933) has demonstrated a similar situation in the mackerel.

The percentage mortality at the different temperatures has been plotted against the stage of development in Fig. 2. The percentage mortality is obtained by dividing the total number of live eggs at the beginning of any 24-hour period into the number of eggs that died in the same period. During the hatching period all eggs which had hatched on previous days were subtracted from the total. The stages of development were interpolated from Fig. 1, and the curves presented here are the mean values for all the jars in each tank. In order to determine accurately the total number of eggs in each jar at the beginning of the experiment, the number of eggs that died daily and the number that hatched were added together. These totals are tabulated in Table I.

Special mention must be made of the 8° C. curve. The first batch of eggs all died on the first day due to a failure in the apparatus which caused a sudden rise in temperature. A second lot of eggs which had been held at 2° C. for 24 hours before being placed at 8° C. was used. At this time, they were at a stage corresponding to early blastula (Plate I, Fig. 4). The wide variation which occurred day by day in the various jars in the 8° C. tank seems to indicate an extraneous factor influencing the mortality. The consistency between the individual jars would indicate a factor common to them all. In view of the fact that the curves for the other tanks are consistent among themselves, one should view the curve of the 8° tank with reservations in considering the general implications of this experiment.

An examination of the curves in Fig. 2 of the percentage mortality at 6, 10, and 12° C. shows that there is general agreement in two respects: a decided decrease in mortality during closing of the blastopore and an increase in mortality as hatching is approached. A different way of stating this conclusion is that the susceptibility of the egg to harmful factors in the environment is least between closure of the blastopore and the first signs of muscular movement, which occurs when the embryo occupies approximately three-quarters of the circumference of the egg (Plate II, Fig. 14). The curve for 8° C. shows the same general trend but the large fluctuations appear to have no satisfactory explanation. The important feature to be noted in these curves is their similarity. There is a decline in mortality toward the same stage irrespective of the temperature. To be sure, the length of the period of

low mortality is longest at 6° C., which is near the optimum temperature, but the periods of high mortality are the same for 6° as for the other temperatures. Since greater numbers of eggs die at 12° C., the higher average position of the curve is understandable.

Rollefsen (1932) has shown that a decrease in susceptibility of cod eggs to shock caused by dropping occurred coincident with the closing of the blastopore. This he explained as an increase in the strength of the yolk covering, preventing rupture. Hein (1907, 1911) performed a series of experiments on trout eggs, subjecting them to various types of adverse conditions at different times after fertilization, and found a similar decrease in susceptibility down to closure of the blastopore and an increase just prior to hatching. My results have confirmed these earlier experiments, but it is not possible at the present state of our knowledge to make any statement concerning correlations between susceptibility and ontogenetic stage beyond the gross description of coincident states.

Summary

1. Experiments were carried out at four different temperatures in the supra-optimal range of development for the cod egg.

2. Development from the two-cell stage to 50 per cent hatched required 8.5 days at 12° C., 9 days at 10° C., 11.5 days at 8° C., and 17.2 days at 6° C.

3. At all temperatures an initial period of high mortality decreased with the closing of the blastopore and was followed by a period of low mortality until the embryo was three-quarters the circumference of the egg membranes, when the mortality steadily increased up to hatching.

4. The hatching period is discussed with reference to its use as a stage of development.

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